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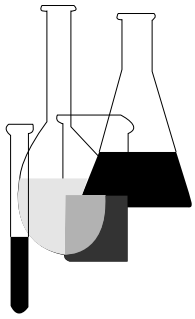
Health Effects Test Guidelines OPPTS 870.6850 Peripheral Nerve Function



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on disks or paper copies: call (202) 512–0132. This guideline is also available electronically in PDF (portable document format) from EPA's World Wide Web site (http://www.epa.gov/epahome/research.htm) under the heading ''Researchers and Scientists/Test Methods and Guidelines/OPPTS Harmonized Test Guidelines.''

OPPTS 870.6850 Peripheral nerve function.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)(7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA)(15 U.S.C. 2601).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline are 40 CFR 798.6850 Peripheral Nerve Function and OPP 85–6 Peripheral Nerve Function (Pesticide Assessment Guidelines, Subdivision F—Hazard Evaluation; Human and Domestic Animals, Addendum 10, EPA report 540/09–91–123, March 1991).

(b) **Purpose.** In the assessment and evaluation of the potential human health effects of substances, it may be necessary to test for neurophysiological effects. Substances that have been shown to produce peripheral neuropathy in other neurotoxicity studies (or other neuropathological changes in peripheral nerves), as well as substances with a structural similarity to those causing such effects, may be appropriate to evaluate with this test. This guideline defines procedures for evaluating certain aspects of the neurophysiological functioning of peripheral nerves. Our purpose is to evaluate the effects of exposures on the velocity and amplitude of conduction of peripheral nerves. Any observed effects should be evaluated in the context of both the concordance between functional neurological and neuropathological effects and with respect to any other toxicological effects seen. Additional tests may be necessary to completely assess the neurophysiological effects of any substance.

(c) **Definitions.** The definitions in section 3 of the Toxic Substances Control Act (TSCA) and the definitions in 40 CFR Part 792—Good Laboratory Practice Standards apply to this test guideline. The following definitions also apply to this test guideline.

Amplitude is the voltage excursion recorded during the process of recording the compound nerve action potential. It is an indirect measure of the number of axons firing.

Conduction velocity is the speed at which the compound nerve action potential traverses a nerve.

Neurotoxicity is any adverse effect on the structure or function of the nervous system related to exposure to a chemical substance.

(d) **Principle of the test method.** The test substance is administered to several groups of experimental animals, one dose being used per group. The peripheral nerve conduction velocity and amplitude are assessed using electrophysiological techniques. The exposure levels at which significant neurotoxic effects are produced are compared to one another and to those levels that cause neuropathological effects and/or other toxic effects.

(e) **Test procedures**—(1) **Animal selection**—(i) **Species and strain.** Testing should be performed on a laboratory rodent unless such factors as the comparative metabolism of the chemical or species sensitivity to the toxic effects of the test substance, as evidenced by the results of other studies, dictate otherwise. All animals should have been laboratory-reared to ensure consistency of diet and environmental conditions across groups and should be of the same strain and from the same supplier. If this is not possible, groups should be balanced to ensure that differences are not systemically related to treatment.

(ii) **Age and weight.** Young adult animals (42–120 days old for rats) should be used.

(iii) **Sex**. In order to reduce the number of animals used, and because of the labor-intensive nature of this testing, only one sex may be used. If data indicate that one sex is more sensitive to the test substance, or if it receives greater exposure, it may be preferred. If females are used, they should be virgins.

(2) **Number of animals.** At least 10 animals should be used in each test and control group. The number of animals to be used should be based on appropriate statistical methods and an allowance for attrition due to anticipated problems, such as loss due to anesthesia, etc. Animals should be randomly assigned to treatment and control groups. If not, some justification is required.

(3) **Control groups.** (i) A concurrent control group is required. For control groups, subjects should be treated in the same way as for an exposure group except that administration of the test substance is omitted.

(ii) Positive control data from the laboratory performing the testing should provide evidence that the experimental procedures are sensitive to substances or procedures known to affect peripheral nerve function. Permanently injurious substances need not be used. Temperature change could be used as a positive control procedure without causing permanent injury to the animals. Historical data may be used if the essential aspects of the experimental procedure remain the same. Periodic updating of positive control data is recommended. New positive control data should also be collected when personnel or some other critical element in the testing laboratory has changed.

(4) **Dose levels and dose selection.** At least three doses should be used in addition to the vehicle control group. The data should be sufficient to produce a dose-effect curve. The Agency strongly encourages the use of equally spaced doses and a rationale for dose selection that will enable detection of dose-effect relations to the highest degree.

(i) **Acute studies.** The high dose need not be greater than 2 g/kg. The high dose should result in significant neurotoxic effects or other clear-

ly toxic effects, but not result in an incidence of fatalities that would preclude a meaningful evaluation of the data. The middle and low doses should be fractions of the high dose. The lowest dose should produce minimal effects, e.g., an ED10, or alternatively, no effects.

(ii) **Subchronic (and chronic) studies.** The high dose need not be greater than 1g/kg. The high dose should result in significant neurotoxic effects or other clearly toxic effects, but not produce an incidence of fatalities that would prevent a meaningful evaluation of the data. The middle and low doses should be fractions of the high dose. The lowest dose should produce minimal effects, e.g an ED10, or alternatively, no effects.

(5) **Route of administration.** Selection of route may be based on several criteria including, the most likely route of human exposure, bioavailability, the likelihood of observing effects, practical difficulties, and the likelihood of producing nonspecific effects. For many materials, it should be recognized that more than one route of exposure may be important and that these criteria may conflict with one another. The route that best meets these criteria should be selected. Dietary feeding will be generally be acceptable for repeated exposure studies.

(6) **Combined protocol.** The test described in this guideline may be combined with any other toxicity study, as long as none of the requirements of either are violated by the combination.

(7) **Study conduct**—(i) **Choice of nerves.** The nerve conduction velocity test must assess the properties of both sensory and motor nerve axons separately. Either a hind limb (e.g. tibial) or tail (e.g. ventral caudal) nerve must be chosen. Response amplitude may be measured in a mixed nerve.

(ii) **Preparation.** (A) In vivo testing of anesthetized animals is required. A barbiturate or an inhalation anesthetic such as isoflorane is appropriate. Care should be taken to ensure that all animals are administered an equivalent dosage and that the dosage is not excessive. If dissection is used, extreme caution must be observed to avoid damage to either the nerve or the immediate vascular supply.

(B) Both core and nerve temperature must be monitored and kept constant (± 0.5 °C) during the study. Monitoring of skin temperature is adequate if it can be demonstrated that the skin temperature reflects the nerve temperature in the preparation under use. Skin temperature should be monitored with a needle thermistor at a constant site, the midpoint of the nerve segment to be tested.

(iii) **Electrodes**—(A) **Choice of electrodes.** Electrodes for stimulation and recording may be made of any conventional electrode material, such as stainless steel, although electrodes made of non-polarizing materials are preferable. If surface electrodes are used, care must be taken to

ensure that good electrical contact is achieved between the electrode and the tissue surface. All electrodes must be thoroughly cleaned following each application.

(B) **Electrode placement.** Electrode placement must be constant with respect to anatomical landmarks across animals (e.g. a fixed number of millimeters from the base of the tail). Distances between electrodes used to calculate conduction velocity must be measurable to ± 0.5 mm. The recording electrodes should be as far from the stimulating electrodes as possible. A 40 mm separation is adequate in the caudal tail nerve of the rat.

(C) **Recording conditions.** (*1*) The animal should be grounded at about midpoint between the nearest stimulating and recording electrodes. With the preamplifier set at its maximal band width, the stimulus artifact should have returned to baseline before any neural response to be used in the analysis is recorded.

(*2*) The electrical stimulator must be isolated from ground. Biphasic or balanced pair stimuli to reduce polarization effects are acceptable. A constant current stimulator is preferred (and required for polarized electrodes) and should operate from about 10 μA to about 10 mA. If a constant voltage stimulator is used, it should operate to 250V. All equipment should be calibrated with respect to time, voltage, and temperature.

(*3*) The testing environment should be isolated from extraneous light and noise and controlled for temperature. Enclosure in a Faraday cage can help reduce 50 Hz noise. The recording output should be amplified sufficiently to render the compound action potential easily measurable with an oscilloscope. The amplifier should pass signals between 2.0 Hz and 4 kHz without more than a 3dB decrement. The preamplifier must be capacitatively coupled or, if direct coupled to the first stages, must be able to tolerate any DC potentials which the electrode-preparation interface produces, and operate without significant current leakage through the recording electrodes.

(*4*) A hard copy for all waveforms or averaged waveforms from which measurements are derived, and for all control recording required by this standard must be available. Hard copies must include a time and voltage calibration signal.

(iv) **Procedure**—(A) **General.** Stimulation should occur at an interstimulus interval significantly below the relative refractory period for the nerve under study. Stimulus intensity should be increased gradually until the response amplitude no longer increases. At this point the maximal stimulus current is determined. An intensity 25–50 percent (a fixed value in a given study) above the maximal intensity so determined should be used for determining response peak latency and response amplitude. Response peak latency may be read off the oscilloscope following single

sweeps or determined by an average of a fired number of responsors. The baseline-to-peak height technique (under paragraph (g)(2) of this guideline) is acceptable for determination of the nerve compound action potential amplitude, but in this case, at least 16 responses must be averaged.

(B) **Motor nerve.** Motor conduction velocity may be measured from a mixed nerve by recording the muscle action potential which follows the compound action potential of the nerve. The stimulus intensity should be adjusted so that the amplitude of the muscle action potential is supramaximal. Measurement of the latency from stimulation to the onset of the compound muscle action potential gives a measure of the conduction time of the motor nerve fibers. To calculate the conduction velocity, the nerve must be stimulated sequentially in two places each with the same cathode-anode distance, and with the cathode located toward the recording electrode. The cathode to cathode distance between the two sets of stimulating electrodes should be divided by the difference between the two latencies of muscle action potential in order to obtain conduction velocity. Placement of electrodes should be described; site of nerve stimulation may differ from point of entry through skin.

(C) **Sensory nerve.** The somatosensory evoked potential may be used to determine the sensory nerve conduction velocity in a mixed nerve. The cathode should be placed proximally at the two stimulation locations with the same cathode-anode distances. The recording electrodes are placed on the skull. The conduction velocity is calculated by dividing the distance between the two stimulating cathodes by the difference between the two latencies of the largest primary peak of the somatosensory evoked potential. Between 64 and 123 responses should be averaged. The stimulation frequency should be about 0.5 Hz. Stimulus intensity should be the same as that used for determining the motor conduction velocity. Should the peak of the somatosensory response be so broad that it cannot be replicated with an accuracy of less than 5 percent of the latency difference observed, then a point on the rising phase of the potential should be chosen, e.g. at a voltage that is 50 percent of the peak voltage. Alternatively, the sensory nerve conduction velocity can be obtained from a purely sensory nerve or from stimulation of the dorsal rootlets of a mixed nerve, using two recording electrode pairs.

(f) **Data collection, reporting, and evaluation.** The final test report must include the following information:

(1) **Description of equipment and test methods.** (i) Give a description of the experimental chambers, programming equipment, data collection devices, and environmental test conditions should be provided.

(ii) Provide a description of the experimental design including procedures for balancing treatment groups.

(iii) Positive control data from the laboratory performing the test which demonstrate the sensitivity of the procedure being used should be provided. Historical data may be used if all essential aspects of the experimental protocol are the same. Historical control data can be critical in the interpretation of study findings. The Agency encourages submission of such data to facilitate the rapid and complete review of the significance of effects seen.

(iv) Include hard copies of waveforms from which measurements were made as well as control recordings.

(v) Provide voltage and time calibration referable to the standards of the National Institute of Standards and Technology (NIST) or to other standards of accuracy sufficient for the measurements used.

(vi) Include data demonstrating that nerve temperature was maintained constant throughout the recording period.

(2) **Results.** Data for each animal should be arranged in tabular form by test group, including the animal identification number, body weight, nerve conduction velocity, and amplitude. Group summary data should also be reported, including standard measures of central tendency and variability, e.g., means and standard deviations, and results of statistical analyses.

(3) **Evaluation of data.** (i) The findings should be evaluated in the context of preceding and/or concurrent toxicity studies and any correlated functional and histopathological findings. The evaluation should include the relationship between the doses of the test substance and the incidence and magnitude of any observed effects, i.e. dose-effect curves for any effects seen.

(ii) The evaluation should include appropriate statistical analyses. Choice of analyses should consider tests appropriate to the experimental design, including repeated measures. There may be many acceptable ways to analyze data.

(iii) Guidance for interpretation of peripheral nerve function data is described under paragraph (g)(5) of this guideline.

(g) **References.** The following references should be consulted for additional background information on this test guideline:

(1) Aminoff, M.J. (Ed.) *Electrodiagnosis in Clinical Neurology.* Churchill Livingstone, NY (1980).

(2) Daube, J. Nerve Conduction Studies. In: *Electrodiagnosis in Clinical Neurology.* M.J. Aminoff (Ed.) Churchill Livingstone, NY. Pp. 229–264 (1980).

(3) Glatt, A.F. et al. Testing of peripheral nerve function in chronic experiments in rats. *Pharmacology and Therapeutics* 5:539–534 (1979).

(4) Johnson, E.W. *Practical Electromyography*. Williams and Wilkins, Baltimore (1980).

(5) U.S. Environmental Protection Agency. Guidelines for Neurotoxicity Risk Assessment. FEDERAL REGISTER 63 FR 26926–26954, May 14, 1998.